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IN THE CANADIAN PATENT OFFICE

Examiner	:	M. Gillen
Applicant	:	Biogen, Inc.
Application No.	:	374,378
Filed	:	April 1, 1981
For	:	DNA SEQUENCES, RECOMBINANT DNA MOLECULES AND PROCESSES FOR PRODUCING HUMAN FIBROBLAST INTERFERON-LIKE POLYPEPTIDES

AFFIDAVIT OF WALTER C. FIERS

EXHIBITS 1-20

SUGANO EXHIBIT 1002
FIERS V. SUGANO
INTERFERENCE NO. 105,661

Opened / Décachetée le: CURRICULUM VITAE 20 02

Commissioner of Patents / Commissaire des brevets

In presence of examiner / en présence de l'examinateur

Walter Charles Cornelius FIERS
Date and place of birth: January 31, 1931, Ieper (Belgium)
Nationality: Belgian
Married; three children

1. Studies

High School: "Koninklijk Atheneum", Ieper, 1949
University: Faculty of Agricultural Sciences, Ghent:
Engineer of Chemistry and Agricultural Sciences, 1954
"Agrégé" for Higher Education (Biochemistry), 1960
PhD, 1963

2. Scientific Career

1954 - 1955	Fellowship of the IWONL
1956	Assistant, Laboratory of Physiological Chemistry, Faculty of Medicine, University of Ghent
	Fellowship of the Danish Government (Danish-Belgian Cultural Agreements)
1957 - 1959	Research Assistant with the NFWO
1960 - 1961	Research Fellowship from the Rockefeller Foundation, New York, USA
1960 - 1961	Research Fellow in Biology, California Institute of Technology, Pasadena, CA, USA
1960 - 1962	Senior Research Assistant with the NFWO
1962	Research Associate, Institute for Enzyme Research, University of Wisconsin, Madison, WI, USA
1963	Assistant Professor at the Faculty of Agricultural Sciences, University of Ghent, Belgium
1967	Associate Professor at the Faculty of Sciences, University of Ghent, Belgium Responsible for the postgraduate course in Molecular Biology
1967	Director-Head of the Laboratory of Molecular Biology, University of Ghent, Belgium
1969	Professor of Molecular Biology at the Faculty of Sciences, University of Ghent, Belgium

3. Residences Abroad

Oct. 1, 1956 - Sept. 30, 1957
Carlsberg Laboratory, Copenhagen, Denmark (with Prof. Dr. H. Holter)
Oct. 1, 1960 - March 30, 1962
California Institute of Technology, Department of Biophysics, Pasadena, CA, USA (with Prof. Dr. R.L. Sinsheimer)
April 1, 1962 - Oct. 1, 1962, and May 1, 1963 - June 7, 1963
Institute for Enzyme Research, University of Wisconsin, Madison, WI, USA (with Prof. Dr. H.G. Khorana)

This is EXHIBIT FIERS-1
to
the Affidavit of Walter C. Fiers
sworn before me
this 13th day of November, 2001

Commissioner for Oath or Notary Public

4. Scientific Awards

1961 - Triannual Award "J.B. Van Helmont" (period 1958-1960) of the Royal Flemish Academy of Medicine of Belgium
1966 - Award of the Flemish Chemical Society (1964-1965)
1971 - Medal of the Société de Chimie Biologique de France
1975 - Award "Doctor A. De Leeuw - Damry - Bourlart" of the National Foundation for Scientific Research of Belgium for the period 1970-1975 (mathematical, physical and chemical sciences)
1976 - Francqui Award (Francqui Foundation, Belgium)
1978 - Doctor honoris causa, Catholic University of Leuven, Belgium
1980 - Jenkinson Memorial Lecture, University of Oxford, UK
1986 - Dr. Beijerinck Gold Medal for Virology, Royal Dutch Academy of Sciences, The Netherlands
- Rik & Nel Wouters Prize for Cancer Research, Belgium
1989 - Arnois - Baillet Latour Prize, Belgium
- Carlos J. Finlay Prize (UNESCO Prize for Microbiology, including Immunology, Molecular Biology and Genetics)
1990 - Personal title of "Baron" and hereditary nobility conferred by H.M. the King of Belgium
1991 - Robert Koch Prize (Robert Koch-Stiftung, Bonn, Germany)

5. Memberships, Offices and Committee Assignments

1966 - Elected to the Council of the Belgian Biochemical Society
- Elected as a member of the European Molecular Biology Organization (EMBO)
1969 - Member of the Advisory Board of the "European Journal of Biochemistry"
1970 - Visiting professor at the Catholic University of Leuven, Belgium
1971 - Member of the European Association for Cancer Research
- Member of the Editorial Board of "Natuur en Techniek", The Netherlands
1972 - Chairman of the research group "Oncoviruses" (formed on behalf of the Higher Council against Cancer), Department of Health, Belgium
- Member of the Editorial Board of "Intervirology"
- Guest member of the Dutch "Working Group on Nucleic Acids" (SON)
1973 - Corresponding member of the Royal Academy of Belgium, Class of Sciences
1974 - Organizer of the EMBO Workshop "Restriction Enzymes and DNA sequences", De Cirkel, Drongen, Belgium
- Member of the International Scientific Committee of the "International Institute of Cellular and Molecular Pathology" (ICP), Brussels, Belgium
- Member of the Scientific Board of the Department of Molecular Biology, ULB, Brussels, Belgium
1975 - Member of the Liaison Committee for Recombinant DNA Research of the European Science Foundation
- Member of the NFWO Commission for Biochemistry and Molecular Biology
1976 - Member of the Council for Medical Ethics, Foundation for Medical Scientific Research of Belgium
1977 - Member of the Editorial Board of "Gene"
- Member of the Scientific Council for Cancer Research of the ASLK
- Member of the Council of the European Molecular Biology Organization
- Member of the Overseas Advisory Panel of "The Biochemical Journal"

1978 - Second vice-president of the Belgian Biochemical Society
1979 - "Chaire Francqui" at the State University of Liège, Belgium
- Member of the IUPAB Commission on Subcellular and Macromolecular Biophysics
- Member of the Programme Committee for the 5th International Congress for Virology
- Member of the Editorial Board of "Biochimie"
- Member of the Scientific Board of Biogen Inc.
1980 - Member of the Advisory Panel of the NATO Advanced Study Institutes
1981 - Member of the Royal Academy of Belgium, Class of Sciences
1982 - President of the Scientific Council for Cancer Research of the ASLK
- Member of the Editorial Board of "The EMBO Journal"
- President of the Belgian Biochemical Society
1983 - Member of the Editorial Board of "Nucleic Acids Research"
- Member of the Editorial Board of "Anticancer Research"
- Commander in the Order of Leopold
1984 - Member of the National Committee for Biochemistry
1985 - President of the NFWO Commission for Biochemistry and Molecular Biology (until 1990)
1987 - Member of the Commission for Biotechnology, Flemish Council for Science Policy
- Member of the Editorial Board of "Biotherapy"
1988 - Member of the FEBS Fellowship Committee
- Member of the Editorial Board of "The European Journal of Immunology"
- Member of the Editorial Board of "Molecular Biology Reports"
- Member of the Editorial Board of "Biotechnology Therapeutics"
- Member of the Editorial Board of "Methods in Molecular and Cellular Biology"
- Member of the Cell Board Subcommittee of the "Medical Research Council" (UK)
- Member of the EEC Study Group on Ethical, Social and Legal Aspects of the Predictive Medicine Programme
1989 - Member of the Editorial Board of "Cytokine"
- Member of the "Academia Europaea"
- Elected member of "The Human Genome Organisation" (HUGO)
- Member of the Board of the "Foundation van Gysel for Medical Research"
1990 - President of the Scientific Council for Cancer Research of the ASLK-Insurances
- Corresponding member of the "American Association for Cancer Research"
- Member of the Scientific Steering Committee for the EMBL (appointed by the Flemish Executive)
- Belgian representative to the Council of the "International Society for Interferon Research" (ISIR)
- Civil Cross First Class
1991 - Member of the "Scientific Advisory Committee" (SAC) for the EMBL (appointed by the EMBL Council)
- Member of the Editorial Board of "Cancer Communications"
- Honorary member of the Royal Flemish Society of Engineers
- Member of the Scientific Board of the "Institut Pasteur du Brabant"
1992 - Member of the Editorial Board of "International Journal of Oncology"
- Member of the Scientific Council of the "International Institute of Cellular and Molecular Pathology" (ICP), Brussels, Belgium
- Honorary member of the Royal Academy of Medicine of Belgium
- President of the "4th International Congress on Tumor Necrosis Factor and Related Cytokines", organized in Veldhoven, The Netherlands

"Chaire Francqui" at the Catholic University of Louvain (KUL), Faculty of Medicine, Belgium

1994 - Member of the Editorial Board of "Circulatory Shock"

1995 - Member of the Editorial Board of "Lymphokine and Cytokine Research"

- Member of the Editorial Board of "Natural Immunity"

- Member of the Editorial Board of "The Journal of Inflammation"

1996 - Grand Officer in the Order of the Crown

- Retired as Professor at the University of Ghent and became Professor emeritus

- Director of the VIB (Flanders Interuniversity Institute for Biotechnology), Department of Molecular Biology

Dec 30 2002

Walter FIERS

Commissioner of Patents
Commissaire des brevets

Date of examination
Date de l'examination

LIST OF PUBLICATIONS

A. Research papers

B. Short communications

C. a. Books (contributed chapters, editorships)

b. Reviews concerning own research

c. General (molecular biology, virology, etc.)

D. Abstracts

This is EXHIBIT FIERS-2
to
the Affidavit of Walter C. Fiers
sworn before me
this 19 th day of November, 2001

Commissioner for Oath or Notary Public

A. RESEARCH PAPERS

A.1 FIERS, W. and STOCKX, J.
The chromatographic separation of mixtures of diglycerophosphoric acids and related products.
Bull. Soc. Chim. Belg. 65, 937-941, 1956.

A.2 FIERS, W. and MÖLLER, K.M.
A micro-method for the determination of ribonuclease activity.
Compt. rend. Lab. Carlsberg 31, 507-510, 1960.

A.3 FIERS, W. and VANDENDRIESSCHE, L.
Catabolism of nucleosides by barley-extracts.
Arch. Intern. Physiol. Biochim. 68, 303-307, 1960.

A.4 FIERS, W.
The determination of ribonuclease activity.
Anal. Biochem. 2, 126-139, 1961.

A.5 FIERS, W. and VANDENDRIESSCHE, L.
The ribonuclease-activity of barley.
Arch. Intern. Physiol. Biochim. 69, 339-363, 1961.

A.6 FIERS, W. and DE BERSAQUES, J.
The reaction mechanism of a nucleoside phosphorylase.
Enzymologia 24, 197-210, 1962.

A.7 FIERS, W. and SINSHEIMER, R.L.
The structure of the DNA of bacteriophage ΦX174. I.
The action of exopolynucleotidases.
J. Mol. Biol. 5, 408-419, 1962.

A.8 FIERS, W. and SINSHEIMER, R.L.
The structure of the DNA of bacteriophage ΦX174. II.
Thermal inactivation.
J. Mol. Biol. 5, 420-423, 1962.

A.9 FIERS, W. and SINSHEIMER, R.L.
The structure of the DNA of bacteriophage ΦX174. III.
Ultracentrifugal evidence for a ring structure.
J. Mol. Biol. 5, 424-434, 1962.

A.10 FIERS, W.
Een mikromethode voor de bepaling van ribonuklease, en toepassingen.
Verhand. Kon. Vl. Acad. Geneesk. Belg. 25, 337-381, 1963.

A.11 FIERS, W. and KHORANA, H.G.
Studies on polynucleotides. XXII. Enzymic degradation. An exonuclease from Lactobacillus acidophilus R 26. A. Purification, properties, and substrate specificity.
J. Biol. Chem. 238, 2780-2788, 1963.

A.12 FIERS, W. and KHORANA, H.G.
Studies on polynucleotides. XXIII. Enzymic degradation. An exonuclease from Lactobacillus acidophilus R 26. B. Stepwise degradation of oligonucleotides.
J. Biol. Chem. 238, 2789-2796, 1963.

A.13 FIERS, W., LEPOUTRE, L. and VANDENDRIESSCHE, L.
Studies on the bacteriophage MS2. I. Distribution of purine sequences in the viral RNA and in yeast RNA.
J. Mol. Biol. 13, 432-450, 1965.

A.14 FIERS, W., DE WACHTER, R., LEPOUTRE, L. and VANDENDRIESSCHE, L.
Studies on bacteriophage MS2. II. Mono-, di- and trinucleotide composition of the pancreatic ribonuclease digest of MS2 RNA.
J. Mol. Biol. 13, 451-462, 1965.

A.15 DE WACHTER, R. and FIERS, W.
Detection of uridine 3'-ethyl phosphate and homologues in digests of ribonucleic acid hydrolyzed with pancreatic ribonuclease in the presence of traces of ethanol.
Arch. Intern. Physiol. Biochim. 74, 741-746, 1966.

A.16 FIERS, W., VERPLANCKE, H. and VAN STYVENDAELE, B.
The synthesis of bacteriophage MS2 RNA in vitro.
In Koningsberger, V.V. and Bosch, L. (eds.), "Regulation of Nucleic Acid and Protein Biosynthesis", Elsevier Publishing Company, Amsterdam, pp. 156-166, 1967.

A.17 DE WACHTER, R. and FIERS, W.
External standardization in liquid scintillation counting of homogeneous samples labeled with one, two, or three isotopes.
Anal. Biochem. 18, 351-374, 1967.

A.18 FIERS, W.
Studies on the bacteriophage MS2. III. Sedimentation heterogeneity of viral RNA preparations.
Virology 33, 413-424, 1967.

A.19 DE WACHTER, R. and FIERS, W.
Studies on the bacteriophage MS2. IV. The 3'-OH terminal undecanucleotide sequence of the viral RNA chain.
J. Mol. Biol. 30, 507-527, 1967.

A.20 MOLEMANS, F., VAN MONTAGU, M. and FIERS, W.
Model substrates for the rapid assay of specific ribonuclease activity.
Eur. J. Biochem. 4, 524-529, 1968.

A.21 VANDENBERGHE, A., VAN STYVENDAELE, B. and FIERS, W.
Studies on the bacteriophage MS2. V. The nucleoside 5'-triphosphate end groups of the replicative intermediate and the replicative form.
Eur. J. Biochem. 7, 174-181, 1969.

A.32 MIN JOU, W. and FIERS, W.
Studies on the bacteriophage MS2. VII. Structure determination of the longer polypurine sequences present in the pancreatic ribonuclease digest of the viral RNA.
J. Mol. Biol. 60, 197-201, 1969.

A.33 DE WACHTER, R. and FIERS, W.
Sequences at the 5'-terminus of bacteriophage Qß RNA.
Nature 221, 233-235, 1969.

A.34 GILLIS, E., DE BOEVER, J.C. and FIERS, W.
The factor-dependence of bacteriophage Qß RNA-polymerase.
Arch. Intern. Physiol. Biochim. 77, 548-550, 1969.

A.35 SLEGERS, H. and FIERS, W.
Studies on bacteriophage MS2. VIII. Evidence for dimer formation of MS2 RNA by reaction with formaldehyde at acidic pH.
Biopolymers 8, 1373-1388, 1970.

A.36 DE WACHTER, R. and FIERS, W.
Fractionation of RNA by electrophoresis on polyacrylamide gel slabs.
In Grossman, L. and Moldave, K. (eds.), "Nucleic Acids", Methods in Enzymology, Vol. 21, Part D, Academic Press, New York-London, pp. 181-178, 1971.

A.37 CROSS, H.J., DUERINCK, F.R. and FIERS, W.C.
The tRNA pyrophosphorylase activity of Escherichia coli. A study on substrate specificity.
Eur. J. Biochem. 17, 116-123, 1970.

A.38 HAEGEMAN, G., MIN JOU, W. and FIERS, W.
Studies on the bacteriophage MS2. IX. The heptanucleotide sequences present in the pancreatic ribonuclease digest of the viral RNA.
J. Mol. Biol. 27, 597-613, 1971.

A.39 DE WACHTER, R., VANDENBERGHE, A., HERREGAERT, J., CONTRERAS, R. and FIERS, W.
The loader sequence from the 5'-terminus to the A-protein initiation codon in MS2-virus RNA.
Proc. Natl. Acad. Sci. USA 68, 383-389, 1971.

A.40 DE WACHTER, R., HERREGAERT, J., VANDENBERGHE, A., CONTRERAS, R. and FIERS, W.
Studies on the bacteriophage MS2. The untranslated 5'-terminal nucleotide sequence preceding the first cistron.
Eur. J. Biochem. 23, 409-414, 1971.

A.41 DE WACHTER, R. and FIERS, W.
Preparative two-dimensional polyacrylamide gel electrophoresis of ^{32}P-labeled RNA.
Anal. Biochem. 49, 184-197, 1972.

A.42 MIN JOU, W., HAEGEMAN, G., ysebaert, M. and FIERS, W.
Nucleotide sequence of the gene coding for the bacteriophage MS2 coat protein.
Nature 237, 82-88, 1972.

A.43 REMAUT, E. and FIERS, W.
Studies on the bacteriophage MS2. XVI. The termination signal of the A protein cistron.
J. Mol. Biol. 71, 221-241, 1972.

A.44 VANDAMME, E., REMAUT, E., VAN MONTAGU, M. and FIERS, W.
Studies on the bacteriophage MS2. XVII. Suppressor-sensitive mutants of the A protein cistron.
Mol. Gen. Genet. 119, 329-336, 1972.

A.45 VERBRAEKEN, E. and FIERS, W.
Studies on the bacteriophage MS2. XX. Expansion of the virion in low salt.
Virology 50, 898-900, 1972.

A.46 CONTRERAS, R., YSEBAERT, M., MIN JOU, W. and FIERS, W.
Bacteriophage MS2 RNA: Nucleotide sequence of the end of the A protein gene and the intercistronic region.
Nature New Biology 241, 99-101, 1973.

A.47 SLEGERS, H. and FIERS, W.
Studies on the bacteriophage MS2. XXIII. Conformation of MS2 RNA in acid medium.
Biopolymers 12, 2007-2021, 1973.

A.48 SLEGERS, H. and FIERS, W.
Studies on the bacteriophage MS2. XXIII. Fixation of the MS2 RNA acid structure by formaldehyde.
Biopolymers 12, 2023-2033, 1973.

A.49 SLEGERS, H., CLAUWAERT, J. and FIERS, W.
Studies on the bacteriophage MS2. XXIV. Hydrodynamic properties of the native and acid MS2 RNA structures.
Biopolymers 12, 2811-2844, 1973.

A.50 HAEGEMAN, G. and FIERS, W.
Studies on the bacteriophage MS2. An internal nucleotide frequent resembling some ribosomal binding sites.
Eur. J. Biochem. 36, 113-143, 1973.

A.51 VOLCKAERT, G. and FIERS, W.
A simple and highly sensitive method for sequence determination of 32P-labeled oligonucleotides.
Anal. Biochem. 62, 573-583, 1974.

A.52 VAN DE VOORDE, A., ROGIERS, R., VAN HERREWEGHE, J., VAN HEUVERSWYN, H., VOLCKAERT, G. and FIERS, W.
Deoxynucleotide substitutions: A new technique for sequence analysis of RNA.
Nucl. Acids Res. 1, 1053-1067, 1974.

A.53 CONTRERAS, R. and FIERS, W.
A method for the isolation of cytidylate carrier from ribonuclease T1-oligonucleotides.
Anal. Biochem. 67, 319-315, 1975.

A.54 YANG, R., DANNA, K., VAN DE VOORDE, A. and FIERS, W.
Location of the small restriction fragments, Hind-L, Hind-M and Hpa-E, on the Simian Virus 40 genome.
Virology 68, 348-363, 1975.

A.45 VANDENBERGHE, A., MIN JOU, W. and FIERS, W.
5'-Terminal nucleotide sequence (n = 261) of bacteriophage MS2 RNA.
Proc. Natl. Acad. Sci. USA 72, 3528-3563, 1975.

A.46 FIERS, W., CONTRERAS, R., DUERINCK, F., HAEGEMAN, G., MERREGAERT, J., MIN JOU, W., RAEYMAEKERS, A., VOLCKAERT, G., YSEBAERT, M., VAN DE KERCKHOVE, J., WOLF, F. and VAN MONTAGU, M.
A-protein gene of bacteriophage MS2.
Nature 256, 273-278, 1975.

A.47 DEVOS, R., GILLIS, E. and FIERS, W.
The enzymic addition of poly(A) to the 3'-end of RNA using bacteriophage MS2 RNA as a model system.
Eur. J. Biochem. 62, 401-410, 1976.

A.48 YANG, R.C., VAN DE VOORDE, A. and FIERS, W.
Cleavage map of the Simian-Virus-40 genome by the restriction endonuclease III of Haemophilus aegyptius.
Eur. J. Biochem. 61, 101-113, 1976.

A.49 YANG, R.C., VAN DE VOORDE, A. and FIERS, W.
Specific cleavage and physical mapping of Simian-Virus-40 DNA by the restriction endonuclease of Arthrobacter luteus.
Eur. J. Biochem. 61, 119-132, 1976.

A.50 VOLCKAERT, G., MIN JOU, W. and FIERS, W.
Analysis of 32P-labeled bacteriophage MS2 RNA by a mini-fingerprinting procedure.
Anal. Biochem. 72, 433-436, 1976.

A.51 FIERS, W., CONTRERAS, R., DUERINCK, F., HAEGEMAN, G., ISERENTANT, D., MERREGAERT, J., MIN JOU, W., MOLEMANS, F., RAEYMAEKERS, A., VAN DEN BERGHE, A., VOLCKAERT, G. and YSEBAERT, M.
Complete nucleotide sequence of bacteriophage MS2 RNA: Primary and secondary structure of the replicase gene.
Nature 260, 500-507, 1976.

A.52 VAN ROY, F. and FIERS, W.
Mycoplasma in African Green Monkey kidney cell cultures: Biochemical detection and effects in virus-infected cells.
In Vitro 12, 357-363, 1977.

A.53 DEVOS, R., VAN EMMELO, J., BEURINCK-GROSMER, C., GILLIS, E. and FIERS, W.
Addition of ATP: RNA adenyltransferase from Escherichia coli of 3'-linked oligo(A) to bacteriophage QB RNA and its effect on RNA replication.
Biochim. Biophys. Acta 447, 315-327, 1976.

A.54 MIN JOU, W. and FIERS, W.
Studies on the bacteriophage MS2. XXXIII. Comparison of the nucleotide sequence in related bacteriophage RNAs.
J. Mol. Biol. 101, 1047-1068, 1976.

A.55 VAN DE VOORDE, A., CONTRERAS, R., ROGIERS, R. and FIERS, W.
The initiation region of the SV40 VP1 gene.
Cell 9, 117-120, 1976.

A.56 VOLCKAERT, G., CONTRERAS, R., SOEDA, E., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of Simian Virus 40 Hind II restriction fragment.
J. Mol. Biol. 106, 501-510, 1977.

A.57 DERYNCK, R. and FIERS, W.
A two-dimensional electrophoretic procedure for the separation of DNA restriction fragments.
J. Mol. Biol. 110, 307-308, 1977.

A.58 YSEBAERT, M., THYS, F., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the restriction fragments Hind L and Hind M of SV40 DNA.
Nucl. Acids Res. 3, 1431-1441, 1976.

A.59 MIN JOU, W., VAN MONTAGU, M. and FIERS, W.
On the possible modulating role of the isoleucine AUA-Codon in bacteriophage MS2 RNA.
Biochem. Biophys. Res. Commun. 73, 1083-1091, 1976.

A.60 ELTON, R.A. and FIERS, W.
Rhythmic variations in purine run frequencies in bacteriophage RNA.
J. Theoret. Biol. 63, 69-92, 1976.

A.61 DEVOS, R., VAN EMMELO, J., CELEN, P., GILLIS, E. and FIERS, W.
Synthesis by avian-myeloblastosis-virus RNA-dependent DNA polymerase of discrete reverse transcripts of bacteriophage RNA polyadenylated in vitro.
Eur. J. Biochem. 79, 319-333, 1977.

A.62 CONTRERAS, R., VOLCKAERT, G., THYS, F., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the restriction fragment Hind F - Eco RI3 of SV40 DNA.
Nucl. Acids Res. 4, 1001-1014, 1977.

A.63 VAN HEUVERSWYN, H., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the SV40 DNA restriction fragment Hind C - Map 0.
Nucl. Acids Res. 4, 1015-1024, 1977.

A.64 VOLCKAERT, G. and FIERS, W.
A micromethod for base analysis of 32P-labeled oligoribonucleotides.
Anal. Biochem. 83, 228-237, 1977.

A.65 VOLCKAERT, G. and FIERS, W.
Micro thin-layer technique for rapid sequence analysis of 32P-labeled RNA: Double digestion and pancreatic ribonuclease analysis.
Anal. Biochem. 83, 228-249, 1977.

RNAs.
J. Mol. Biol. 126, 1047-1068, 1978.

A.66 CONTRERAS, R., ROGIERS, R., VAN DE VOORDE, A. and FIERS, W.
Overlapping of the VP1-VP3 gene and the VP1 gene in the SV40 genome.
Cell 13, 599-608, 1977.

A.67 HERREGAERT, J., VAN EMMELO, J., DEVOS, R., PORTER, A., FELLNER, P. and FIERS, W.
The 3'-terminal nucleotide sequence of encephalomyocarditis virus RNA.
Eur. J. Biochem. 83, 55-63, 1978.

A.68 VSEBAERT, M., VAN HEUVERSWYN, H., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of part of the Simian Virus 40 Hind-D restriction fragment. The processed initiation region of the VP3 gene.
Eur. J. Biochem. 85, 199-206, 1978.

A.69 ROGIERS, R., VAN DE VOORDE, A., BOEDA, E. and FIERS, W.
Nucleotide sequence of the Simian Virus 40 Hind-K restriction fragment.
Eur. J. Biochem. 85, 305-314, 1978.

A.70 HAEGEMAN, G. and FIERS, W.
Characterization of the 5'-terminal capped structures of late Simian Virus 40-specific mRNA.
J. Virol. 25, 826-830, 1978.

A.71 VAN ROY, F. and FIERS, W.
Interference with Simian Virus 40 DNA replication by Adenovirus type 2 during mixed infection of monkey cells.
J. Virol. 27, 223-227, 1978.

A.72 CONTRERAS, R., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the restriction fragment Hind-F-EcoRII of Simian-Virus-40 DNA (part of the VP1 gene).
Eur. J. Biochem. 86, 317-324, 1978.

A.73 VAN HEUVERSWYN, H., VAN DE VOORDE, A. and FIERS, W.
Complete nucleotide sequence of the Simian-Virus 40 Hind-G fragment and localisation of the carboxyl terminus of the VP1 protein.
Eur. J. Biochem. 86, 325-334, 1978.

A.74 VAN HEUVERSWYN, H., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the Simian-Virus-40 DNA region coding for the carboxyl-terminal part of the T antigen.
Eur. J. Biochem. 86, 335-344, 1978.

A.75 PORTER, A.G., HERREGAERT, J., VAN EMMELO, J. and FIERS, W.
Sequence of 135 nucleotides at the 3'-terminus of encephalomyocarditis virus RNA.
Eur. J. Biochem. 83, 551-561, 1978.

A.76 FIERS, W., CONTRERAS, R., HAEGEMAN, G., ROGIERS, R., VAN DE VOORDE, A., VAN HEUVERSWYN, H., VAN HERREWEGHE, J., VOLCKAERT, G. and YSEBAERT, M.
Complete nucleotide sequence of SV40 DNA.
Nature 273, 113-120, 1978.

A.77 VOLCKAERT, G., VAN DE VOORDE, A. and FIERS, W.
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Proc. Natl. Acad. Sci. USA 75, 2160-2164, 1978.

A.78 VAN ROY, F. and FIERS, W.
Structure of the components of the Adenovirus-Simian Virus 40 hybrid population Ad2+ND2S and reciprocal molecular interactions.
J. Mol. Biol. 124, 691-720, 1978.

A.79 VAN HEUVERSWYN, H., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the Simian Virus 40 Hind II + III restriction fragment 2 and the total amino acid sequence of the major structural protein VP1.
Eur. J. Biochem. 91, 615-630, 1978.

A.80 YSEBAERT, M., VAN DE VOORDE, A. and FIERS, W.
Nucleotide sequence of the Simian Virus 40 Hind II + III restriction fragment D and the total amino acid sequence of the late proteins VP2 and VP3.
Eur. J. Biochem. 91, 631-639, 1978.

A.81 CROSSEAU, H., SANKOFF, D., MIN JOU, W., FIERS, W. and KIDERCREN, B.J.
Bacteriophage MS2 DNA: A correlation between the stability of the codon: anticodon interaction and the choice of code words.
J. Mol. Evol. 12, 113-119, 1978.

A.82 HAEGEMAN, G. and FIERS, W.
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D. ABSTRACTS

Total number: 302.

Dec 30 2002

Commissioner of Patents
Commissaire des brevets

This is EXHIBIT FIERS-3
to
the Affidavit of Walter C. Fiers
sworn before me
this 13 th day of November, 2001

Commissioner for Oath or Notary Public

Deryck Rik 21/4 /

- [illegible] . - 4 ml gelaten op 1 ml GCl { RNA op
-

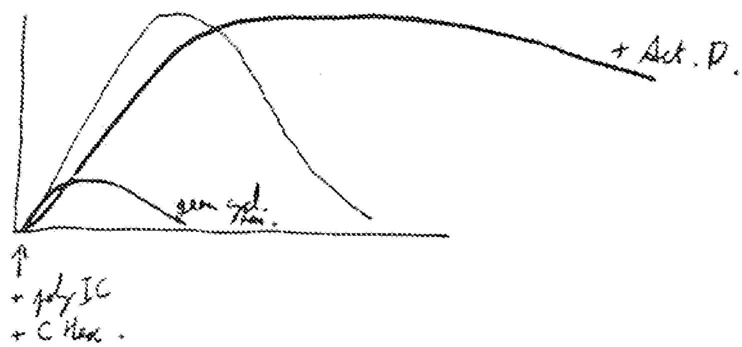
- Zuiver IF $2\text{-}3 \times 10^8$ U/mg.
  - mens, muis -
  - muis: all fibroblast-type.
  - Zuivering - poly U - agarose
    - affigel met specifieke antilich. → gel {35 K / 22 K
    - verbinding 85% 35 K.
    - komt meest actief biol. molecule.
    - Longgel: 2% [illegible] - 2 diffuse banden
    - fingerprints gelijk.
  - Zuiver: [illegible] OK
    - [illegible] niet effief.
- Spec. [illegible]: • Zn - chelaat of Cu - {Fib / Leu.
  - • Blue Sepharose.
- Bolton - Hunter iodinatie: geen verlies v. activiteit
- Nanolose: 1 [illegible] / [illegible] voor [illegible].
- Glycosylatie PAS (periodic acid - Schiff).
  - Reagens 35 K +
  - 22 K -
  - Longgel: gneuraminidase behandelen ⟹ 1 band (IEF).
  - Con A - column: [illegible] 2 pieken - } Leu
  - Tunicamycine: niets meer verbonden → glycosylatie }
    - dus glycosylatie niet essentieel voor biol act.
  - Ongeveer zelfde beeld maar glycosylatie moeilijk volledig te elimineren door tunicamycine behandeling.
  - Iodinatie: verlies v. biol act v. Fib-IF maar moeilijk te bepalen.

- Inductie : poly I:C + DEAE-dextran → goed om mogelijk.

- Productie : Menselijke : meest ook fibr. 3 – 20%
Fibr. : " " leuk. – 1 – 20% (lang
of s. mensen v. inductie.
human interferon; specifiek.
gevormd v. stimulatie v. T lymphocyten.
poly I:C + polylysine : weinig nevenwerkingen – kan als
behandeling aan patiënten toegediend worden.

- mRNA : translatie – mens → X. laevis.
Revel : ook in rat. – minder

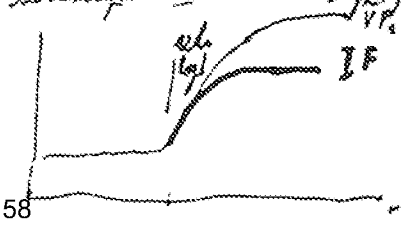
Inductie



Cloning : 4 firma's Searle 1
6 groepen.

- chromosoom : receptor 21
productie: 2 , 5 – rol van 9 !.
- receptor : bind actief. – binding van gelabeld IF
membraanveranderingen in IF behandelde cellen.
- antiviraal effect : verdringt celgroei – niet verandering. morfologie
effect in G_1.
- effect op tumorvirussen : vermindering extracellulair viraal antigen
vermindering intracellulair – defect op maken

- Revel : effect op uncoating ? niet bevestigd – onmiddellijk
IF 10 u na infectie.



tot 6x hogere interne methylatie –   cellulair ook verhoogd.
andere cap –

- Effect in cell-free systeem.

ppp A 2'p 5' A 2'p 5' A = 2'5' A

cell-free systeem : IF + ds RNA + ATP

1) 2'5' A

2) kinase → { Elong.Faktor 2. / histonen

ATP   ds RNA   inact. proteinase

↓ ATP

2'5' A —Factor F→ AMP   act. kinase.

↓   ↳ inhibeert [illegible]

latent endonucl. → actief endonucl.   Euk. Initiator Factor 2 (EI

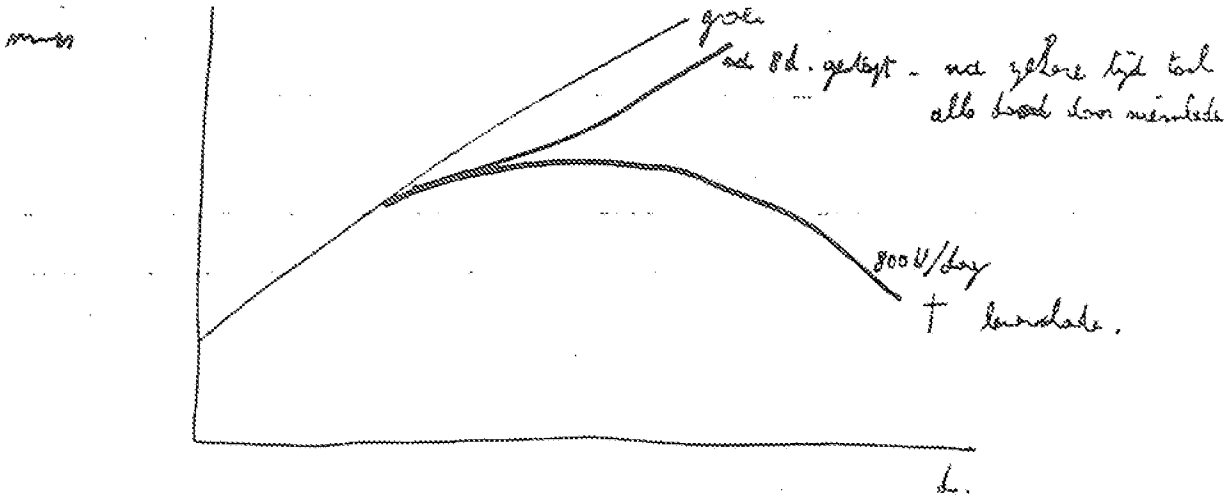
3x stimulatie   Pase   ↓ fosfor (EIF2

{ t RNA methylatie   plaats of rol onzeker

2'5' A ook in normale cellen . verhoging 5x door IF

- Klinisch : L 1210 → L 1210 B (IF resistent – lager receptor).

↓ tumor IF gevoelig   ↓ tumor IF → celkultuur – terug resistent



max $2-5 \times 10^5$ U/kg.

veel trials, meer 99% leucocyten –
+ Herpes bij oog.
eventueel geen positief effect indien ook andere behandeling (bv. steroiden!).
- Ebola
- Hepatitis B –
- Osteosarcoma. Steroiden.
verlenging tot 65-75%

{ Steroiden kunnen nadelig zijn
Niet op kleine dieren.
Kweeken meer sensitief.

Nieuwste: – IF – is equivalent aan leucocyt.

Fibroblast: { miastheniaplastici
hepatitis B –
oog herpetitis

{ fibroblast: groen leeft.
leucocyten: niet "

Nevenwerkingen: koorts: ontsteking?
via prostaglandine?
leidt tot bloedarmoede (minder met FIF)

BIOGEN S.A.

Reply To
Suite 3700
One New York Plaza
New York, New York 100

December 1, 1978

Prof. Dr. W. Fiers
Laboratorium voor
Moleculaire Biologie
9000 Ghect
BELGUIM

Dear Dr. Fiers:

This will confirm our meeting at 8 o' clock a.m. in Ghent on Thursday, December 14. I will have with me Deborah Masters who is one of Biogen's vice presidents. We will meet you at the Ghent railway station. We need to take a 11 o' clock train from Ghent in order to catch the 11:43 a.m. train to Paris.

I look forward to seeing you on the 14th.

Sincerely,

Daniel DL Adams
President &
Managing Director

DDA:bjb

This is EXHIBIT FIERS-4
to
the Affidavit of Walter C. Fiers
sworn before me
this 19th day of November, 2001

Commissioner for Oath or Notary Public

11b Avenue de la Porte-Neuve, Luxembourg

"AR"
9/2, 179
11ème

BIOGEN SCIENTIFIC BOARD MTG. (PARIS)

Robin Nicholson
Ken Murray
Bernard Mach
Ray Schaeffer
Moshe Alafi
Bob Lucianno
Wally Gilbert

Charles Weissmann
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Walter Fiers
Dag Laurason
Brian Hartley
Philippe Kourilsky
Phil Sharpe

Opened Dec 30 2002
Decachetée le

Commissioner of Patents
Commissaire des brevets

In presence of examiner
en présence de l'examinateur

This is EXHIBIT FIERS-5
to
the Affidavit of Walter C. Fiers
sworn before me
this 19th day of November, 2001

Commissioner for Oath or Notary Public

WALTER FIERS

done in collaboration w/ 2 other groups

1- in Louvain

Resomer no longer active

exp. in assay for interf.
prepare cells – winning bioassays

2- Content

Pasteur Institute of Brussels
all translation work.

- Mol. Virol - wg Fiers group in Ghent

Start of fibroblast RNA - gradient → partial purif. – ~ 40 x purif.

~ 150 clones most screening in this area
then 20,000 clones

Using dADT tails (similar compared to C.W. work) - higher level of transformants - ∴ used this for higher yields -

* Techniques of cloning / Some Dr. Chas. Weissman

Detection of screening:
Hybrid arrested Translation —
wanted to be less dependent on posit. artifacts.

System has not worked - despite apparent functioning of controls -

Assays -
① Orig - same as C.W. -
some tech. key content in Seconds

②: Reticulocyte lys.
meas. biol. activity

Much more active in #1 than #2 (~ 10x)

Screened 150 clones in groups of 50 –
mixed results.

HAT - -

out of protein = same whether
or not you want it. ∴ see that system works.

Satellite Tobacco Necrosis Virus
(STNV) is used as marker. (?)

Goat anti-interferon ~~[illegible]~~ also
brings down STNV.

very good messenger
in terms of activity

Either sys. works or
→
· interf. or behaves in suspicious way

Screening 20,000 in groups of 50

Opened Dec 30 2002
Decachetée le

Commissioner of Patents
Commissaire des brevets

In presence of
en présence de l'examinateur

This is EXHIBIT FIERS-6
to
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sworn before me
this 19th day of November, 2001

Commissioner for Oath or Notary Public

(micros. in ml)

- De positieve resultaten op DBM-cellulose zijn zeer duidelijk, ook op herhaling (behalve voor B).

  Gel ④ bevat ook kloon 13, die vroeger twijfelachtig positief scoorde. nu ook duidelijk ⊖.

- De keuze voor verdere grootschalige valt op C.
  - ⊕ op cellulose (2x).
    ⊕ op biologische aktiviteit. (plaque reduction assay ?, maar zijn voorlopig nog geen resultaten)
  - ⊕ op Sephacryl ⇒ andere afwerking
    ⇒ dit kolomgebonden DNA is verder gezuiverd over sukrose gradient.
    (of cellulose uit: bevat nog erg veel RNA)
  - geen enkele maal ⊖ gescoord!

C = 2'/12 (A→D) 46 kloons

19/10

1. opgroeien van tray 2'/12 op dish - LB/agar/tet.
2. enten van prekulturen LB/tet [$A_2 \to C_1$ / $C_2 \to D_{12}$]
3. enten van 400 ml kulturen LB/tet [$A_2 \to C_1$ / $C_2 \to D_{12}$] →(tot. 9.2 l)
4. lyse-procedure

   na afcentrifugeren v.d. kulturen (GS-3, 10 min - 3000 rpm) (3 l per pot)
   2x wassen met 1x TES

   heropnemen in 100 ml sukrose 10% - Tris 50 mM pH 8
   + 10 ml lysozyme (50 mg/ml Tris 25 mM pH 8) (± 30 min)
   + 10 ml EDTA (0.5 M pH 8)
   + 80 ml TLR (over de middag)

   alles bij kamertemperatuur.

   Clearing spin : 6 poly-allomers (40 ml) per groep (→ 3 groepen)
   24 K - 45 min.

5. PEG-precipitatie SN van Clearing spin + 1/3 vol | 40% PEG / 2M NaCl

   overnacht in koele kamer (ext of ijs)

2 : van 1
3 : van 2
4 : eluïne

→ geen aspecifiek plakken !

Het inwezen rel pellets (na opgroei) is wschl de oorzaak van de aanwezigheid van RNA in de DNA-preparaten na lyse en CsCl gradiënt-centrifugatie !

C - subgroepen $\boxed{2^1 - 12}$

C_1 :	$A_2 \to A_9$	(8)
C_2 :	$A_{10} \to A_{11}$, $B_1 \to B_6$	(8)
C_3 :	$B_7 \to B_{12}$, C_1	(7)
C_4 :	$C_2 \to C_9$	(8)
C_5 :	$C_{10} \to C_{12}$, $D_1 \to D_5$	(8)
C_6 :	$D_6 \to D_{12}$	(7)

[redacted] [illegible])

7. CsCl - evenwichts - gradientcentrifugatie (2 per groep . totaal 12)

R60Ti
40K
18°C
over weekend. → banden goed gescheiden < onderdelen vh RNA

8. Aftappen met grosse punktie -> 5 ml tuvno spuit

IAA extractie
+ 3 vol H_2O [illegible]
+ 1/10 vol NaAc 2M pH 5.1
+ 2 vol EtOH   overnacht -20°C

9. EtOH pellets afdraaien

heropnemen in 1/10 STE
fenoliseren - fenolfase wassen
etherextractie 3x
ether verjagen

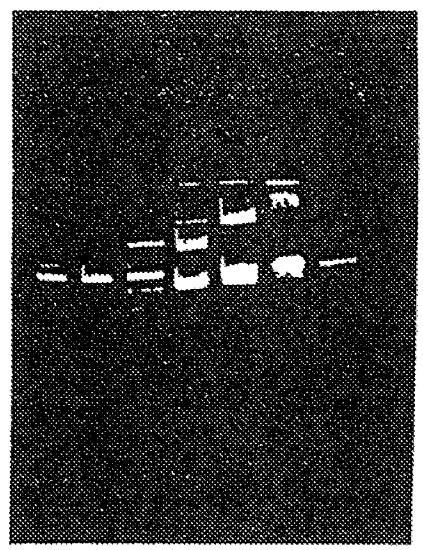
tot vol per groep - 1 ml 1/10 STE

↘ 2 µl op gel laden (1% agarose)
+ 1 µg pBR 322 referentie.

10. Sukrose - snelheidsgradient - centrifugatie

↓

wegens afwezig zijn vh RNA
=> NIET NODIG



1 2 3 ↓ 4 5 6

pBR-322 referentie

↓

=> (0.3) (0.3) (0.4) ±1µg (1) (0.7) (0.8)

SUBGROEPEN C

O_2 : $A_{10} \to A_{11}$, $B_1 \to B_6$ (6)

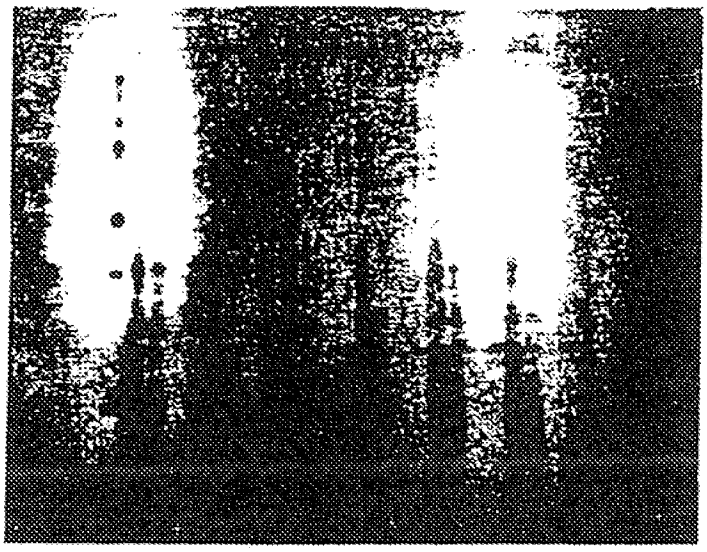
O_3 : $B_7 \to B_{12}$, C_1 (7)

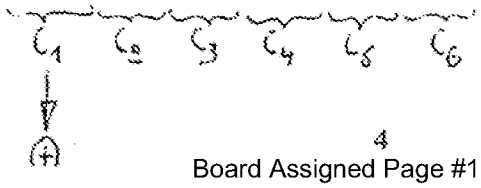
O_4 : $C_2 \to C_9$ (9)

O_5 : $C_{10} \to C_{12}$, $D_1 \to D_5$ (8)

O_6 : $D_6 \to D_{12}$ (7)

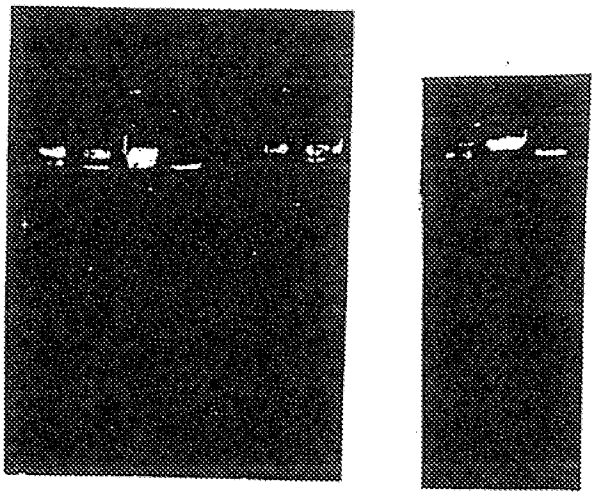
Rezultatul pe coloana C_6





SUBGROEPEN O

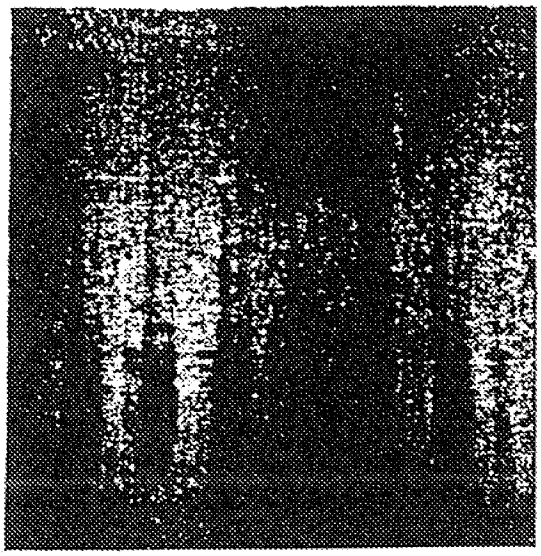
1 2 3/4 5/21 - 5 6

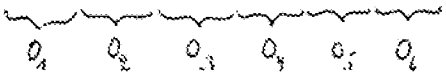


→ 1,4 1,3 1,9 (1,0) 1,2 1,3 1,0 1,3 (1,0)

Er is een weinig RNA aanwezig (→ 3+4 scheen geen RNA, 5 en 6 apart eiwit niets meer)

3,4,5 bevatten chromosomaal DNA.





gekweerd STNV-RNA → 7H weom-gel

3,4. → jaartal-banden, STNV-RNA na CIP-behandeling. (calf-intestine-phosphatase)

5,6,7,8,9 → jaartal-banden, STNV-RNA, zonder voorbehandeling.

(In gevalle-volgorde).

28/11 RNA bereidingen (Rega - Leuven)

- W_1 : non induced VGS # 23, 10 rollers (10/10/'79)
- W_2 : induced VGS # 24, 20 rollers (24/10/'79)
- W_3 : induced VGS # 26, 15 rollers (16/11/'79).

↳ overgebracht als EtOH precipitaat.

* afcentrifugeren 9000 tpm HB4 -20°C

heropnemen TE + 0.1% SDS. 400 µl

\+ 1 vol $CHCl_3$ / IAA
\+ 1 vol fenol en fenolfase nawassen met 50 µl TE (0.1%)

SN 400 + 50 poolen en nawassen met 1 vol $CHCl_3$ / IAA (verwijderen van fenol)

* EtOH precipiteren

↓

29/11 heropnemen in 600 µl TE, 0.1% SDS.

↓ ↘ -20°C

2 µl staaltje, OD meten.

W_1 :	0.101 × 2 × 100 →	20.2 OD/ml	× 0.6 → 12.12 OD	× 40 → 485
W_2 :	0.144	28.8 OD/ml	17.28 OD	691
W_3 :	0.150	30.0 OD/ml	18.00 OD	720

onderverdeeld in batches

- W_1 : 3 × 170 µg
- W_2 : 4 × 20 µg + 4 × 115 µg
- W_3 : 4 × 30 µg + 4 × 132 µg

↳ alles EtOH precipiteren i.a.v. 0.2 M Na Ac. pH 5.1

/ Methode /

F - IF

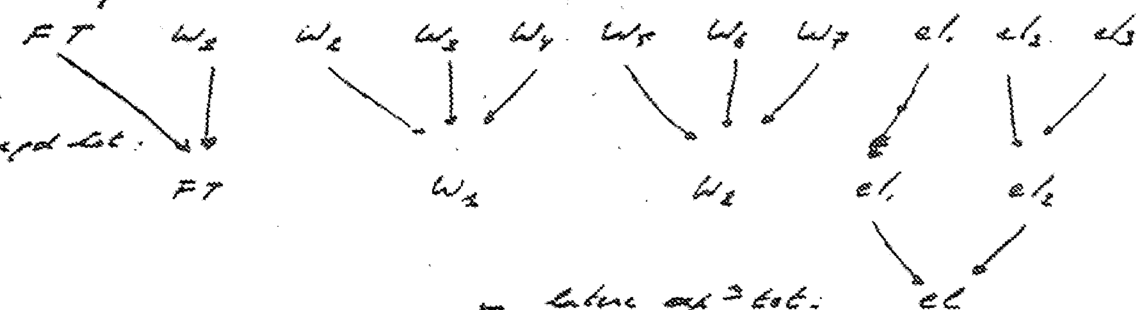
10/4/80

DBM - cellulose ~ DNA
hybridisatie met ... µg
/ 0.05 µg
hybridisatie overnacht
daarna 7x wassen
3x elutie
alles gebeurt batchwise in eppendorfjes (centrifugeren)

Opened Dec 30 20 02
Décachetée le
STNF-RNA
Commissioner of Patents
Commissaire des brevets
In presence of examiner
en présence de l'examinateur

Dus per hybridisatie hebben we

Deze worden samengevoegd tot:



→ later ook 3 tot: el

hierbij is FT: doorloop
W: was
el: eluaat

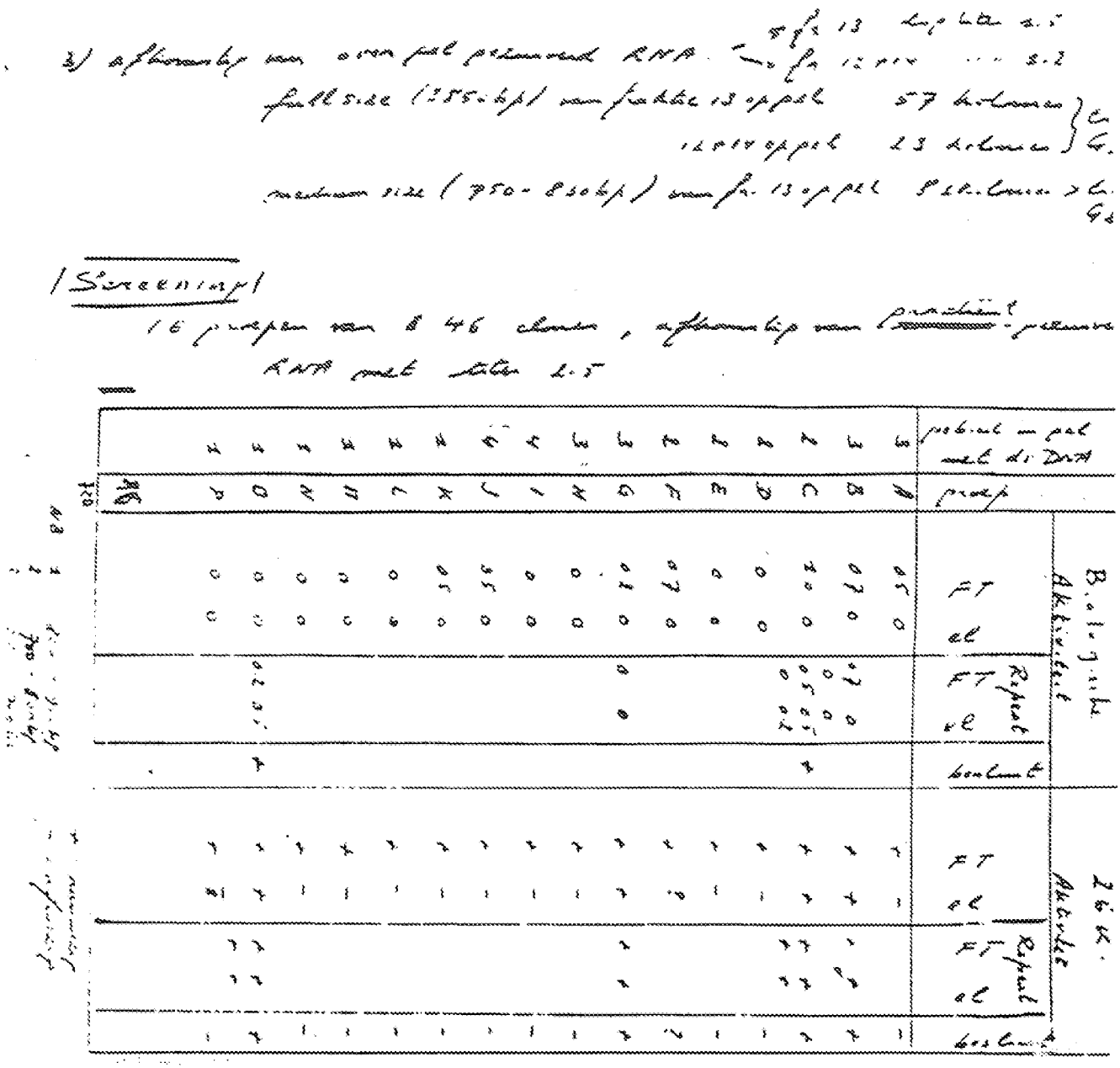
Alle fracties worden na samenvoegen en vóór ethanolprecipitatie verdeeld in 2 helften

(A) 1/2 → precipitatie met bakkersgist tRNA

(B) 1/2 → " " poly A^- RNA (ontkoppeld)

This is EXHIBIT FIERS-7
to
the Affidavit of Walter C. Fiers
sworn before me
this 13 th day of November, 2001

Commissioner for Oath or Notary Public



Board Assigned Page #175

subproefen

voor verder onderzoek kwamen proefen C en O in aanmerking

monster	[illegible]	FT	el	Repeats FT	el	beoord.	FT	el	Repeats FT	el	[illegible]
C_1	8	0	0	0.7	0	–	–	+	–	+	+
C_2	8	0.7	0			–	+ spanjes				–
C_3	7	0.7	0			–	+	–			–
C_4	8	0	0	0	0		+	–			–
C_5	8	0.5	0			–	+	–			–
C_6	7	0	0	1.2	0	–	+	–			–
O_1	8	0	1.2	0 / 0	1.5 / .05	+	+	–	+ / +	– / –	[illegible]
O_2	8	0.7	0			–	+	–			[illegible]
O_3	7	0	0	0 / 1.3	0 / 0	–	–	+	–	+	+
O_4	8	0	0	0.5	0		–	+			+
O_5	8	0.5	0			–	+	–			–
O_6	7	0	0				+	–			–

NB Gezien dat in alle gevallen van subproefen voor 26K afwezig alles in FT of in de positieve proefen alles in el. hadden, mogen we wellicht besluit dat het analoog zal zijn van biologische activiteit.
Daarom worden de subproefen met de biol. akt in FT ook als negatief (–) beschouwd, dit in tegenstelling met de proefen + 48 C ... waar we duidelijk onvolledige hybridisatie hebben

Besluit

C_1 zijn positief voor 26K en negatief voor biolakt I.
O_3 " " " "
O_4 " " "

O_1 is negatief voor 26K en positief voor biolakt II
en ook geen andere banden (≠ 26K) tenzij na zetic + Imm. prec. met anti IF

Individuele klonen

En wordt steeds gevonden met ...

C_8 → 26 K

O_3 → 26 K

O_1 → niet akt. IF.

		aktiviteit FT	aktiviteit cl	inhibit	aktiviteit FT	aktiviteit cl	inhibit
C_8	1	0.1	0	–	+	–	[illegible]
	2	1	0	–	+	–	–
	3	0.7	0	–	+	sup.	
	4	1	0	–	–	+	+
	5	1.2	0	–	+	sup.	
	6	1.2	0	–	+	–	–
	7	.0	0		+	–	–
	8	0	0		+	–	–
O_3	1	niet meer bepaald			+	–	–
	2				+	–	–
	3				+	–	–
	4				+	–	–
	5				+	–	–
	6				+	–	–
	7				–	+	+

NB Gezien 't de [illegible] O_3 en C_8 duidelijk negatief waren voor kool. akt met IF werd geen verder werk meer gestoken [illegible] kool. akt

Besluit $C_8/1$ en $O_3/7$ zijn duidelijk 26K-prod

[illegible]

O_1 is nog de analyse [illegible]

26 K [illegible]

[illegible]

Ab - IF [illegible]

[illegible]

O₁ subgroep

Biologische activiteit

dosis	op DBM-cell. poeder		op DBM-cell. filter		op nitrocellulose filter	
	IF	AL	IF	AL	IF	AL
O₁ 1x	(0.2 / 0.7	0 / 0	0.7	0	1.0	0
2	1.2	0	0.2	0	0.7	0
3	1.2	0	1.0	0.2	1.0	0
4	(1.2 / 0.2	0 / 0	1.0	0	0.2	0
5	0.7	0	0.7	<0.2	[illegible]	0
6	0.7	0	1.0	<0.2	0.5	0
7	0.5	0	1.2	0	<0.2	0.5
8			<0.2	1.2	<0.2	0.7
[illegible]						
O₁ [illegible]	1.2	0.5	<0.2	1.2		

* Deze O. 1? is duidelijk positief voor IF-activiteit.

Dec 30 02

en présence de l'examinateur

This is EXHIBIT FIERS-8
to
the Affidavit of Walter C. Fiers
sworn before me
this 19th day of November, 2001

Commissioner for Oath or Notary Public

after SDS-polyacrylamide gel electrophoresis, the eluate should be centrifuged at 20,000 rev/min (Sorvall SS-34 rotor) for 20 minutes to remove particulate matter before dialysis. Coomassie blue staining of the gels to locate protein bands does not interfere with subsequent sequenator analysis.

New technologies such as the improved amino acid sequencing method described above lead to new research opportunities. With the greater sensitivity provided by this technique, we now can obtain amino acid sequence information on both proteins and peptides with submicrogram (picomole) quantities. This sensitivity should permit analysis of biomedically relevant molecules—such as the interferons—that can only be obtained in microgram quantities, and this ability opens possibilities for further study of these molecules. For example, knowledge of the amino acid sequence permits the synthesis of corresponding DNA probes and opens the possibility of new strategies for isolating genes, such as those for interferons, that express low levels of messenger RNA's (9).

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9. Supported by a gift from the Ben Weingart Foundation.

29 November 1979

Human Fibroblast Interferon: Amino Acid Analysis and Amino Terminal Amino Acid Sequence

Abstract. *The purification of human fibroblast interferon has been simplified to a two-step procedure consisting of affinity chromatography on Blue Sepharose and sodium dodecyl sulfate polyacrylamide gel electrophoresis. A preliminary amino acid composition and the sequence of the 13 amino-terminal residues of homogeneous interferon prepared by this method is reported.*

Since the discovery of interferon, its purification and chemical characterization have been primary goals of interferon research. Although their attainment has been slow because of the small quantities of interferon proteins available, purification to homogeneity has now been achieved with some interferons. However, only microgram quantities have been available for characterization—human fibroblast interferon (*1*, *2*), human lymphoblastoid interferon (*3*), human leukocyte interferon (*4*), murine interferon (*5*)—and only limited structural information has been acquired (*4*, *6*).

A thorough understanding at the molecular level of the numerous phenomena that are caused by interferon in cells in culture and in animals will not be possible until the elucidation of primary and secondary structures of the interferon proteins is achieved. This structural information will permit (i) comparison of amino acid sequences of interferons from various cell types and animal species, (ii) identification of the polypeptide segments involved in binding to interferon-specific cell-surface receptors, and (iii) chemical synthesis of interferons.

We now report an improved procedure for the purification of human fibroblast interferon that can be used to provide enough protein for structural studies. Using the automated protein microsequencing technique described in (*7*), we have determined the sequence of the 13 amino acid residues at the amino terminus of the interferon prepared by this method. We also report a preliminary amino acid composition of the protein.

Human diploid fibroblast cells (FS-4) were cultured and interferon was produced (*1*). Interferon was assayed by a microtechnique (*8*) with vesicular stomatitis virus as the challenge virus. Interferon units are given in National Institutes of Health human fibroblast interferon units.

The crude interferon, 10 to 15 liters produced in the absence of serum, was made 1*M* in NaCl and passed at room temperature through a column (4 by 10 cm) of Blue Sepharose (Pharmacia, Inc.) equilibrated with 0.02*M* sodium phosphate buffer, *p*H 7.2, containing 1*M* NaCl. The interferon was retained whereas more than 95 percent of the total protein passed through the column. The interferon was eluted with a mixture of the column buffer and ethylene glycol (1:1), and each fraction was diluted immediately with 0.5 volume of the buffer (Fig. 1a). Fractions containing interferon activity were pooled, diluted with two volumes of the column buffer, and passed through a small (1 by 6 cm) Blue Sepharose column for concentration. The interferon was eluted as described above (Fig. 1b).

Fractions containing interferon were pooled, dialyzed against 1 m*M* tris-HCl,

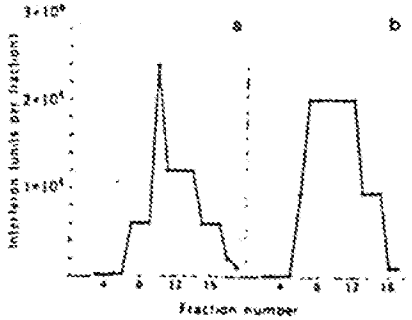

Fig. 1. (a) Fractionation of crude interferon on a large column of Blue Sepharose. Elution of interferon with 50 percent ethylene glycol in column buffer begins at fraction 1. (b) Small Blue Sepharose column. Fractions 7 to 17 in (a) were pooled, passed through the small column, and eluted with 50 percent ethylene glycol in column buffer (fractions 1 to 20).

Table 1. Amino acid composition of human fibroblast interferon.

Amino acid	Composition	
	Mole percent	Residues per 20,000 daltons
Asp	11.3	18.9
Thr	4.0	6.8
Ser	6.2	10.5
Glu	15.9	27.0
Pro	1.6	2.7
Gly*	4.6	7.8
Ala	5.9	10.0
Cys†	1.0	1.7
Val	3.5	6.0
Met	1.7	2.9
Ile	5.3	9.0
Leu	12.0	20.4
Tyr	4.4	7.5
Phe	5.5	9.4
His	2.9	4.9
Lys	6.8	11.6
Arg	6.4	10.9
Trp‡	0.6	1.0

*Includes correction for free glycine present in unhydrolyzed protein. †Determined after performic acid oxidation. ‡Determined after hydrolysis with mercaptoethanesulfonic acid.

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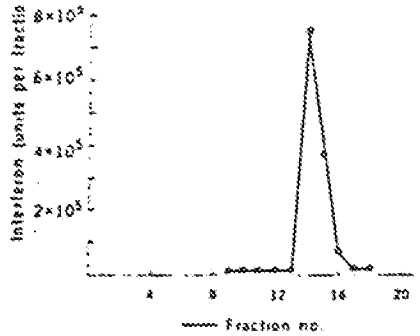

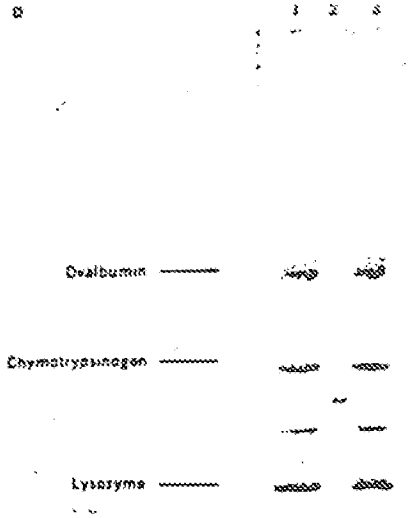

Fig. 2. (a) Preparative electrophoresis of interferon, activity profile. Fractions 6 to 15 in Fig. 1b were pooled, concentrated, and subjected to electrophoresis in a polyacrylamide slab gel, 0.75 mm thick. Fractions 14 and 15 were pooled and processed for amino acid sequencing. (b) Polyacrylamide slab gel, staining of proteins eluted from preparative gel in (a). Approximately 2 percent of the protein in fractions 14 and 15 (a) was subjected to electrophoresis and stained. Lanes 1 and 3, standard proteins; lane 2, interferon.

pH 6.8, containing 0.02 percent sodium dodecyl sulfate (SDS, Bio-Rad electrophoresis grade), and concentrated to dryness in a vacuum centrifuge. The interferon was then subjected to electrophoresis on a SDS-polyacrylamide slab gel and eluted (Fig. 2a). Fractions eluted from the gel were assayed for interferon activity (Fig. 2a). Approximately 0.2 μg of interferon from the peak activity fraction was subjected to electrophoresis in this system again, and the gel was stained with Coomassie blue (Fig. 2b).

The preparative electrophoresis fractions containing interferon were pooled and centrifuged for 30 minutes at 30,000 rev/min at 4°C to remove polyacrylamide gel particles. The interferon solution was dialyzed first against 0.15*M* NaCl containing 0.1 percent SDS and then against 0.02 percent SDS. The dialyzed interferon was concentrated to dryness in a vacuum centrifuge.

This purification procedure is simpler and shorter than that described previously (*1*). Recoveries from the large Blue Sepharose column have ranged from 50 to 100 percent, and those from the small column approach 100 percent. The interferon (5×10^7 U/mg) eluted from these columns is stable for at least 4 weeks at 4°C in 1*M* NaCl, 35 percent ethylene glycol, pH 7.2. Recoveries of activity from the SDS gels have ranged from 5 to 20 percent, and specific activities of this protein have ranged from 2×10^7 to 8×10^7 U/mg. Accurate specific activities are difficult to determine, and two- to fourfold differences above 1×10^8 U/mg are probably not meaningful. Overall yields of purified interferon from 10- to 15-liter batches of crude material (5×10^8 to 7×10^8 total units, 8×10^7 U/mg) have averaged around 10 percent. This gives 5 to 10 μg of homogeneous interferon.

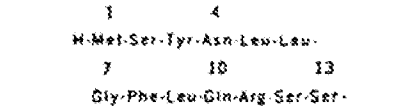

Fig. 3. The amino-terminal amino acid sequence of human fibroblast interferon.

Amino acid analysis on 1- to 2-μg portions was performed on a Durrum D-500 amino acid analyzer (Table 1). Automated Edman degradation on 0.4- to 2-μg portions of the purified interferon was performed on a spinning cup sequenator (*7*). Phenylthiohydantoin (PTH) amino acids were identified by high-performance liquid chromatography (HPLC) on a Du Pont Zorbax CN column (*9*).

The sequence of the 13 amino terminal amino acid residues of human fibroblast interferon was determined by this microsequencing technique (Fig. 3). Yields of PTH methionine at cycle 1 for three sequenator runs ranged from 60 to 100 percent (based on protein determination by amino acid analysis), and the sequenator repetitive cycle yields were 92 to 95 percent. Any unblocked minor peptide sequence present at > 5 percent of the reported sequence could have been detected by the methods used, but none has

homogeneity of the interferon pe
peptide preparation.

Determining the amino acid sequer
of a protein is essential in order to ider
fy its active site and to understand t
molecular mechanism of action. Co
parison of structural features of int
ferons from different species and fr
different cell types within an animal w
prove or disprove whether they are d
ferent proteins. If there is an active s
common to all interferons, it should
identifiable by comparison of the ami
acid sequences. Comparison of the ami
terminal sequence reported here for h
man fibroblast interferon does not as y
reveal any apparent homology with t
amino-terminal sequence reported for h
man lymphoblastoid interferon (*10*
However, there is limited homology (3
13 residues identical) with the 37,00
dalton mouse Ehrlich ascites cell inte
feron (*11*).

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30 November 1979